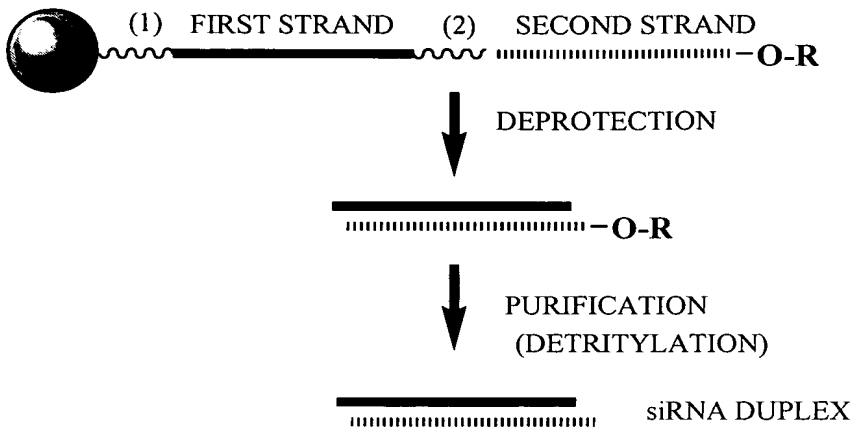
Figure 1

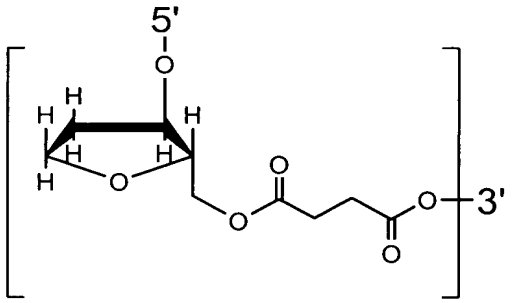


= SOLID SUPPORT

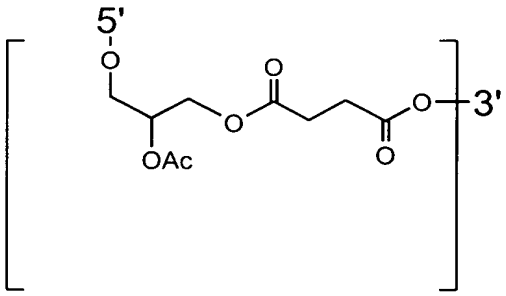
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

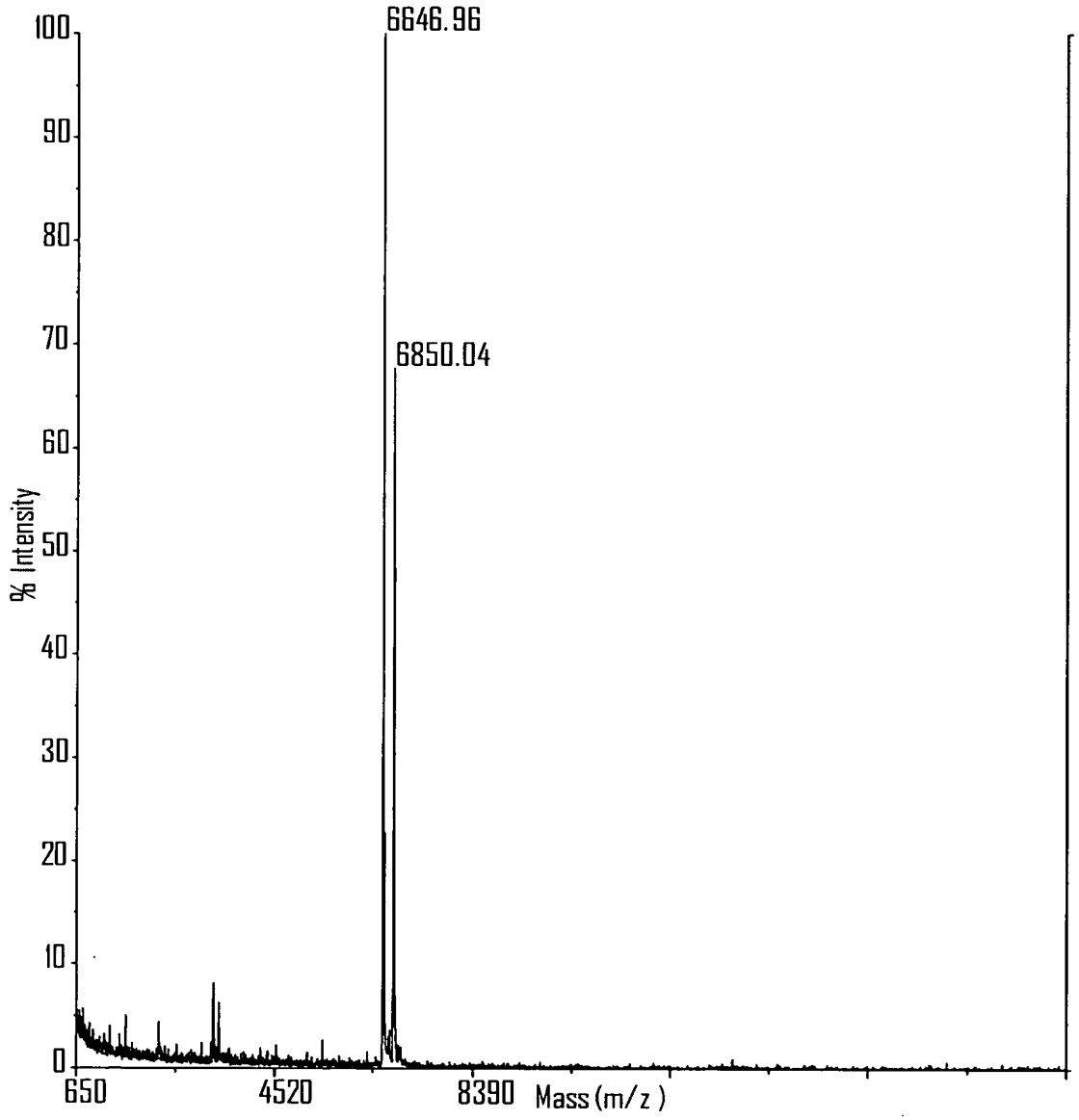

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

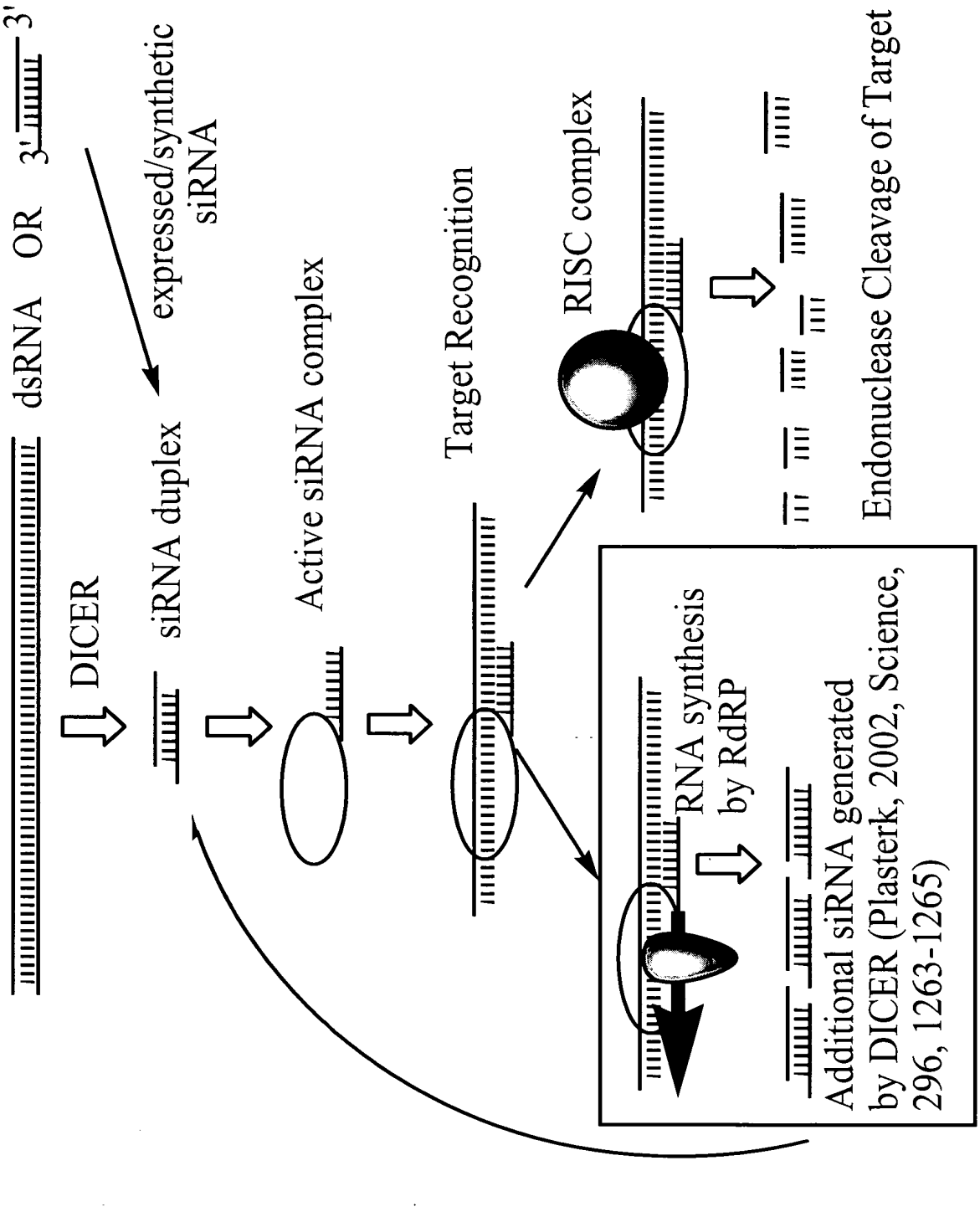

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)
Endonuclease Cleavage of Target

Figure 4

A

SENSE STRAND (SEQ ID NO 703)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 704)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 705)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_sN) -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 706)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 707)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 708)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 709)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

L-(N_sN) N N N N N N N N N N N N N N N N N N N

3'- -5'

ANTISENSE STRAND (SEQ ID NO 706)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 710)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 706)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 709)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 711)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 712)

5'- iB- G C A G C C U A U G G U G U G A G C C *T T*-iB -3'

3'- L-T_S*T* C G U C G G A U A C C A C A C U C G G -5'

ANTISENSE STRAND (SEQ ID NO 713)

B

SENSE STRAND (SEQ ID NO 714)

5'- g *c* a g *c c u* a *u* g g *u* g *u* g a g *c c* T_S*T* -3'

3'- L-T_S*T c* g *u c* g g a *u* a *c c* a *c* a *c u c* g g -5'

ANTISENSE STRAND (SEQ ID NO 715)

C

SENSE STRAND (SEQ ID NO 716)

5'- iB-G G c A G c c u A u G G u G u G A G c c *T T*-iB -3'

3'- L-T_S*T c* G *u c* G G A *u* A *c c* A *c* A *c u c* G G -5'

ANTISENSE STRAND (SEQ ID NO 717)

D

SENSE STRAND (SEQ ID NO 718)

5'- iB-*G G c A G c c u A u G G u G u G A G c c T T*-iB -3'

3'- L-T_S*T c* g *u c* g g a *u* a *c c* a *c* a *c u c* g g -5'

ANTISENSE STRAND (SEQ ID NO 715)

E

SENSE STRAND (SEQ ID NO 719)

5'- iB-G G *c* A G *c c u* A *u* G G *u* G *u* G A G *c c T T*-iB -3'

3'- L-T_S*T c* g *u c* g g a *u* a *c c* a *c* a *c u c* g g -5'

ANTISENSE STRAND (SEQ ID NO 715)

F

SENSE STRAND (SEQ ID NO 718)

5'- iB-*G c A A G A c u c A A G A c A u G A u T T*-iB -3'

3'- L-T_S*T c G u u c u G A G u u c u G u A c u A* -5'

ANTISENSE STRAND (SEQ ID NO 720)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY

iB = INVERTED DEOXYABASIC

L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

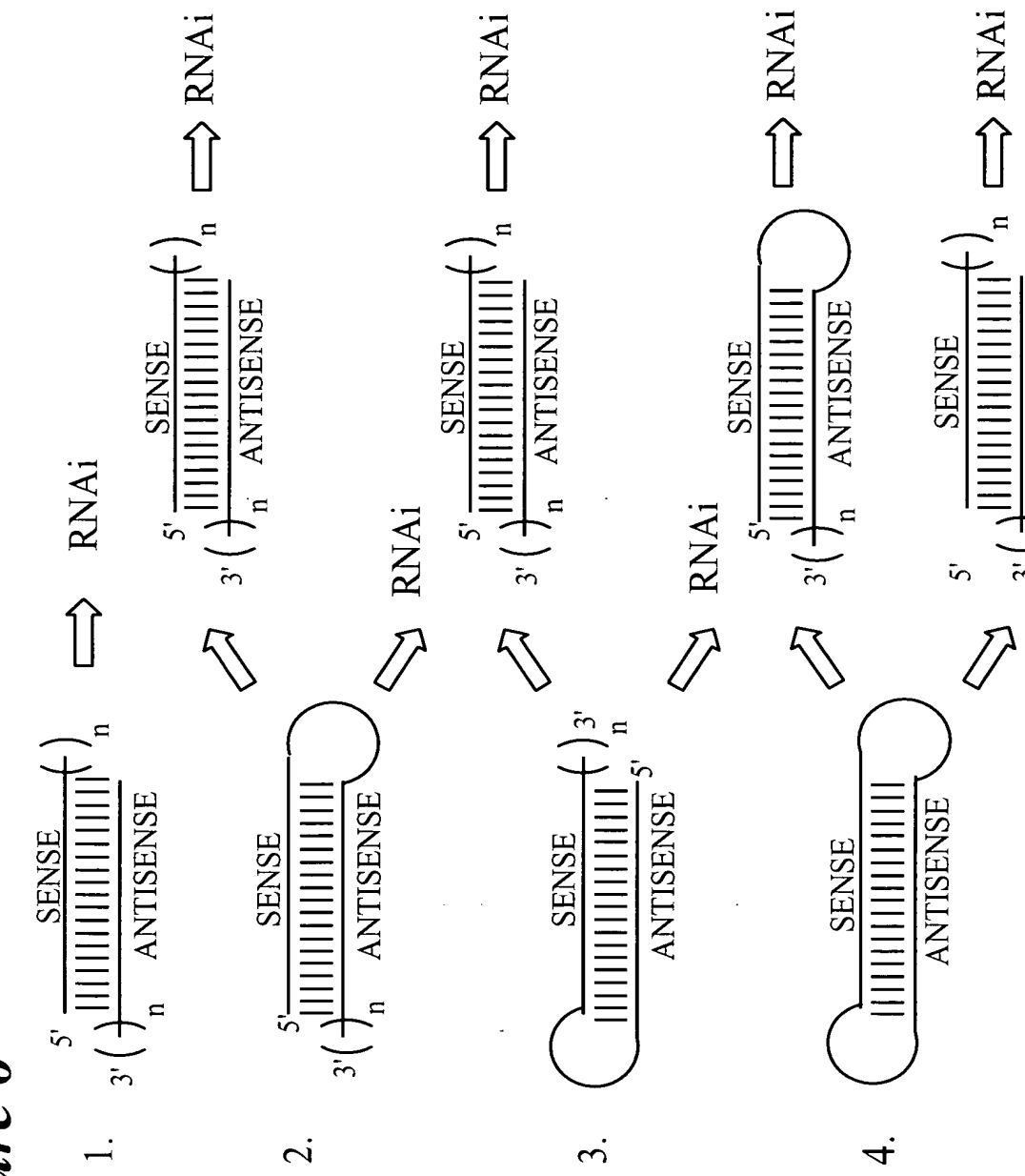


Figure 7

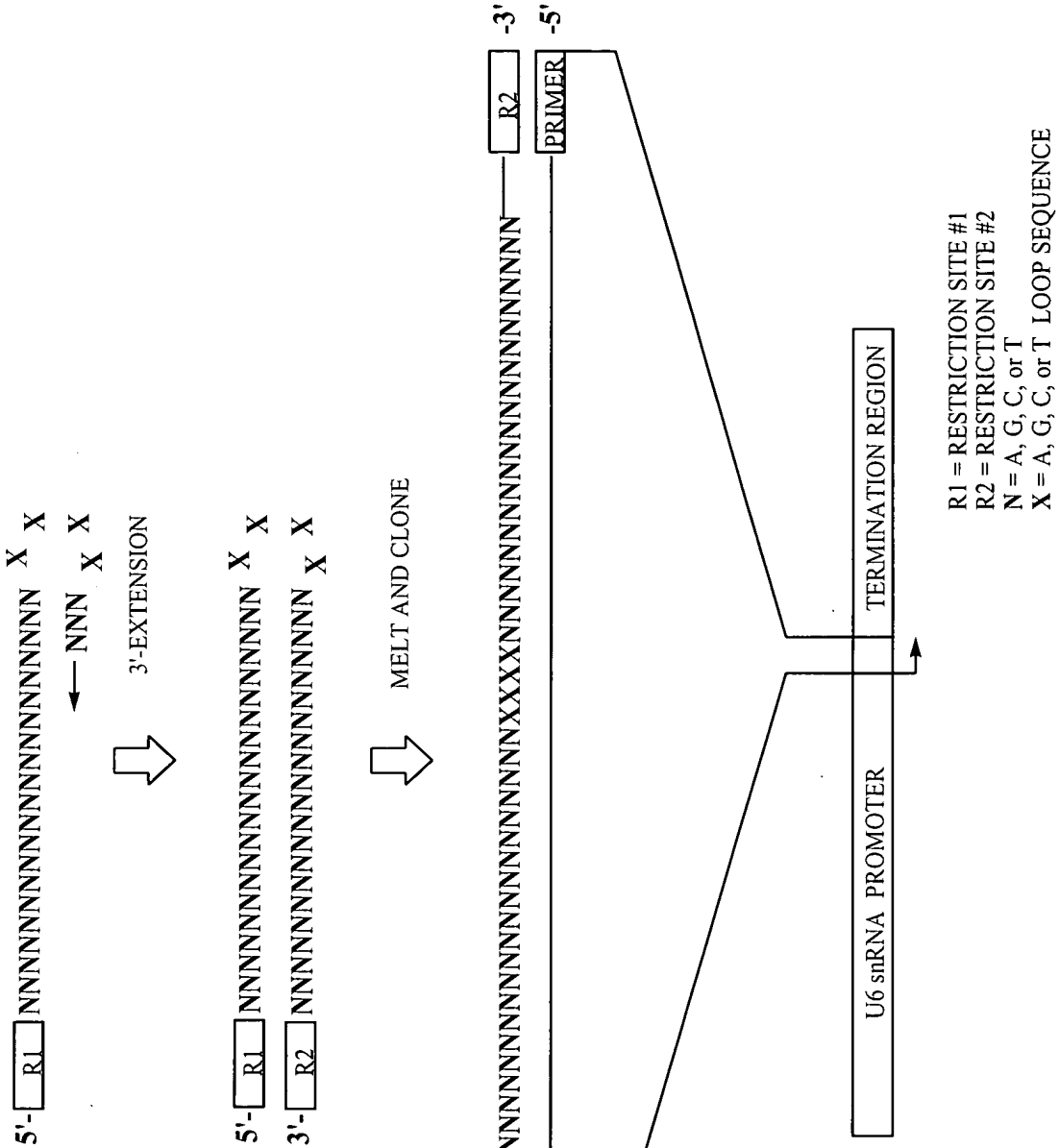

A
5'- R1 NNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 NNNNNNNNNNNNNNNNNNNNNNXXXNNNNNNNNNNNNNNNNNNN R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

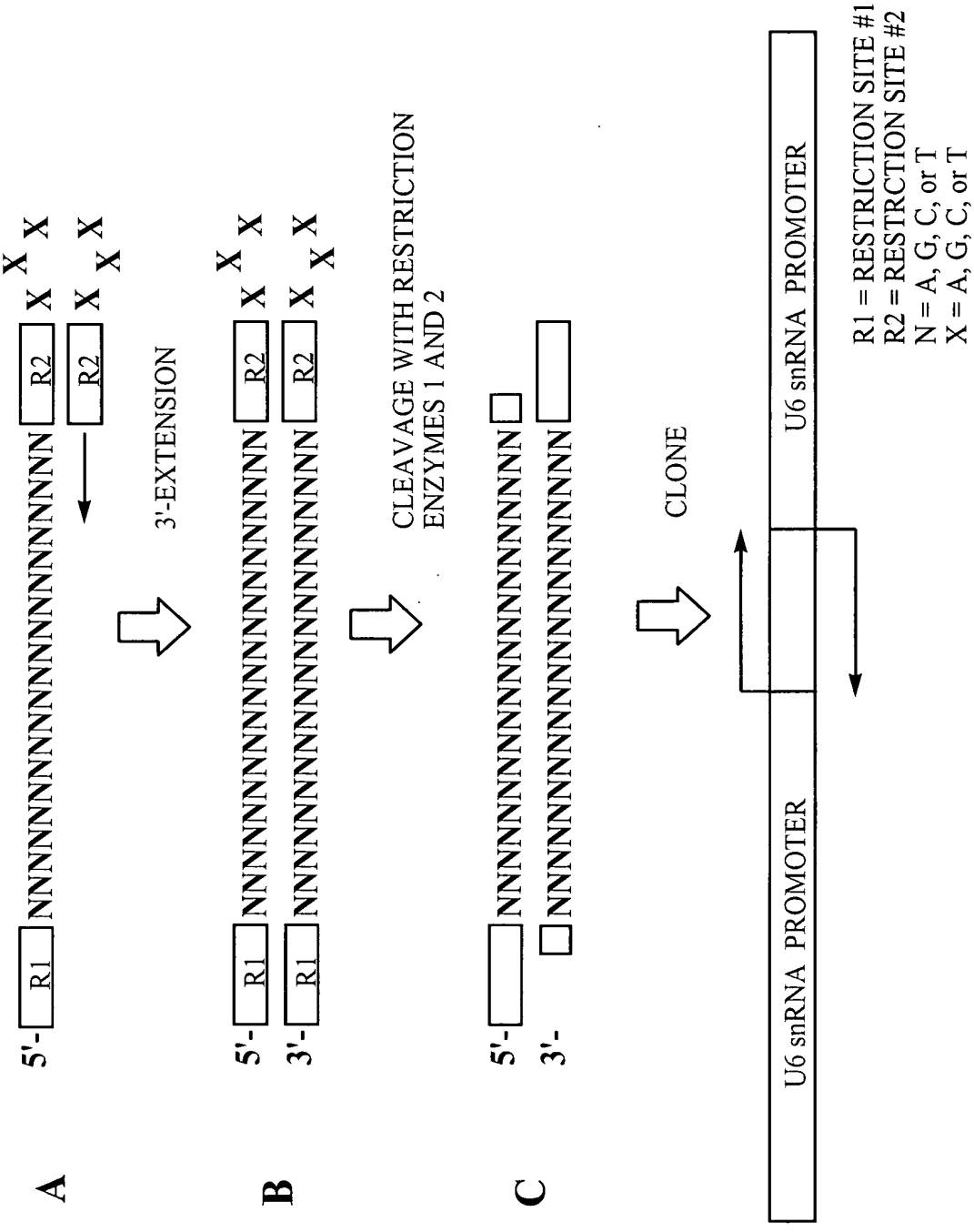

A
5'-
R1
NNNNNNNNNNNNNNNNNNN
R2
X X X X X X
3'-EXTENSION
B
5'-
R1
NNNNNNNNNNNNNNNNNNN
R2
X X X X X X
3'-
R1
NNNNNNNNNNNNNNNNNNN
R2
X X X X X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'-
NNNNNNNNNNNNNNNNNNN
3'-
NNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

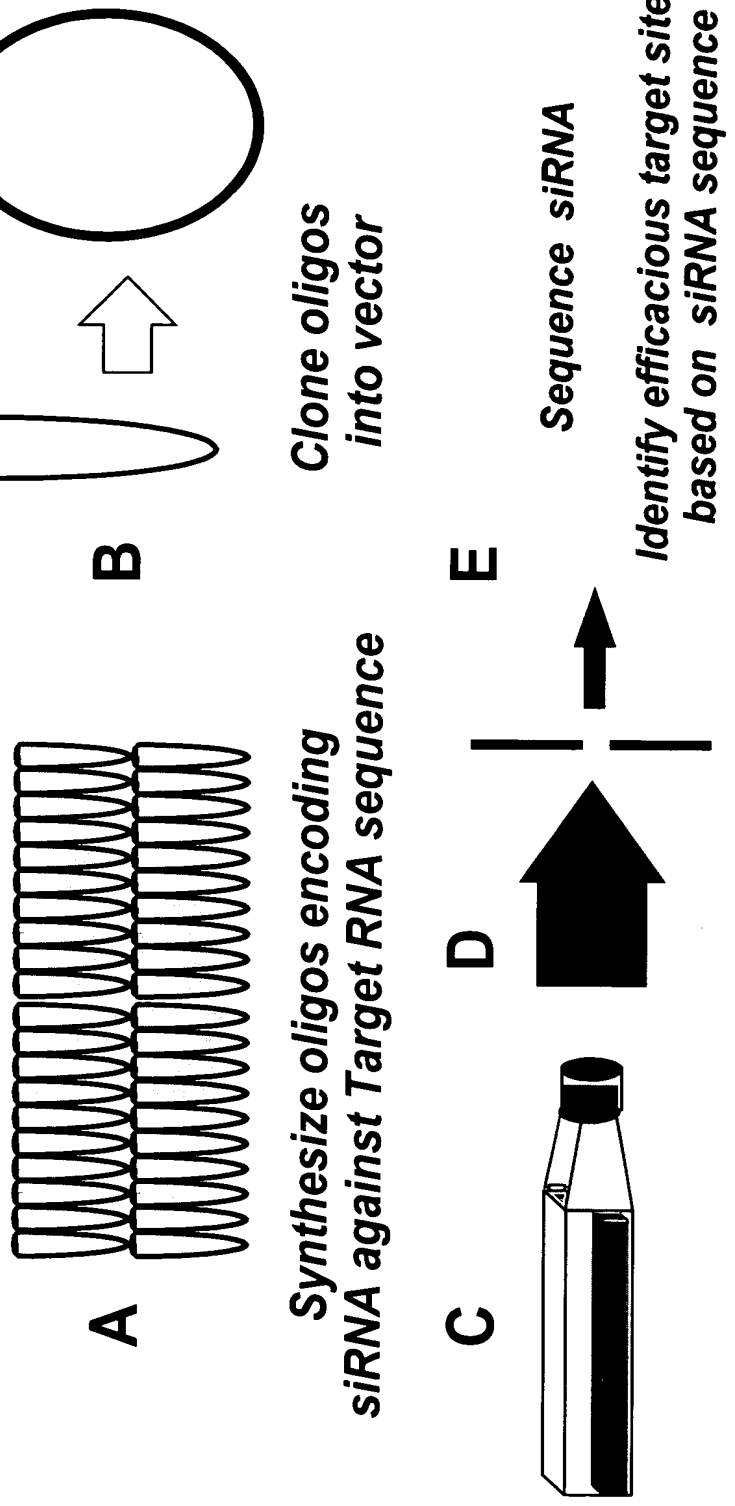
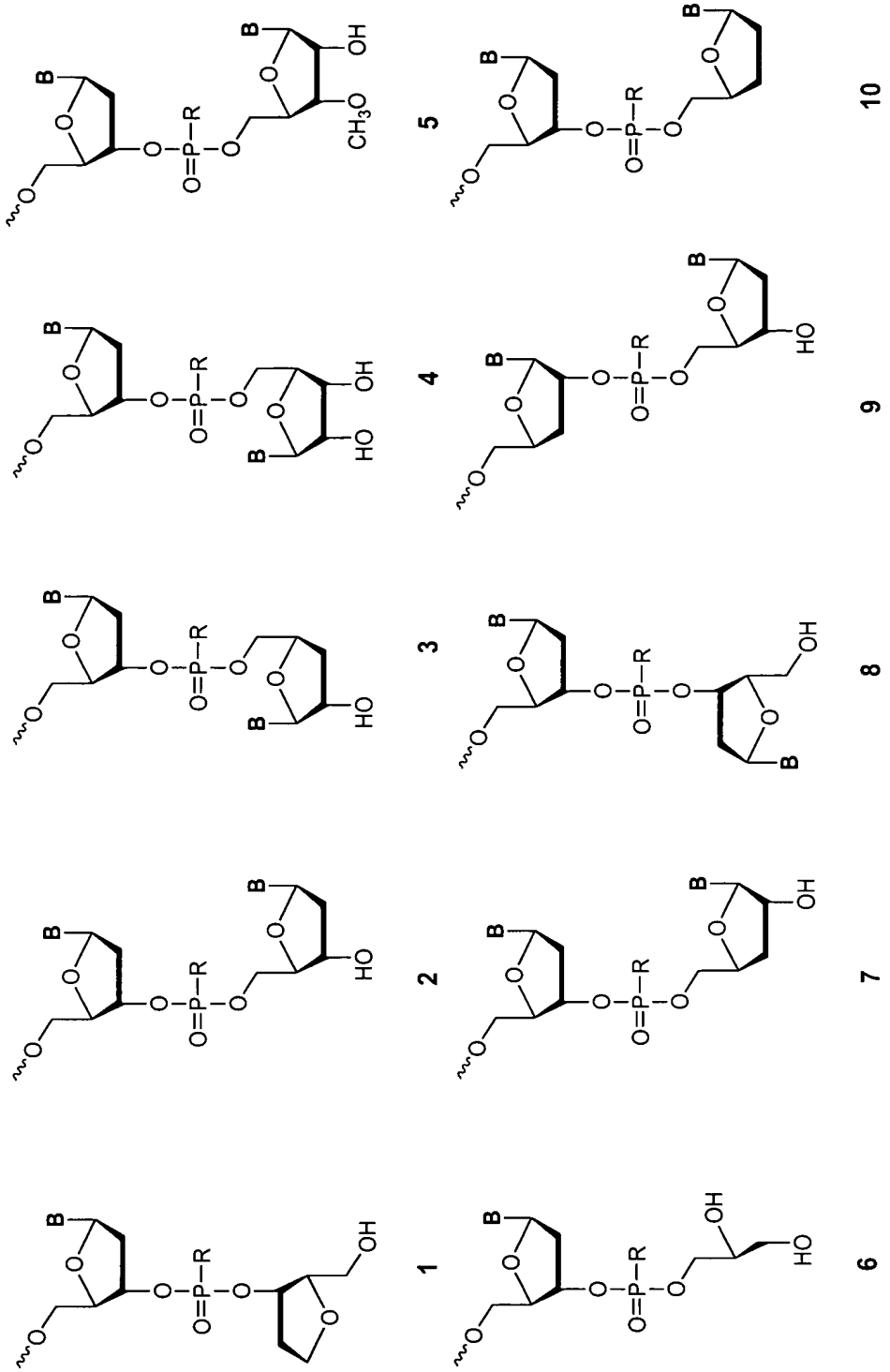


Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

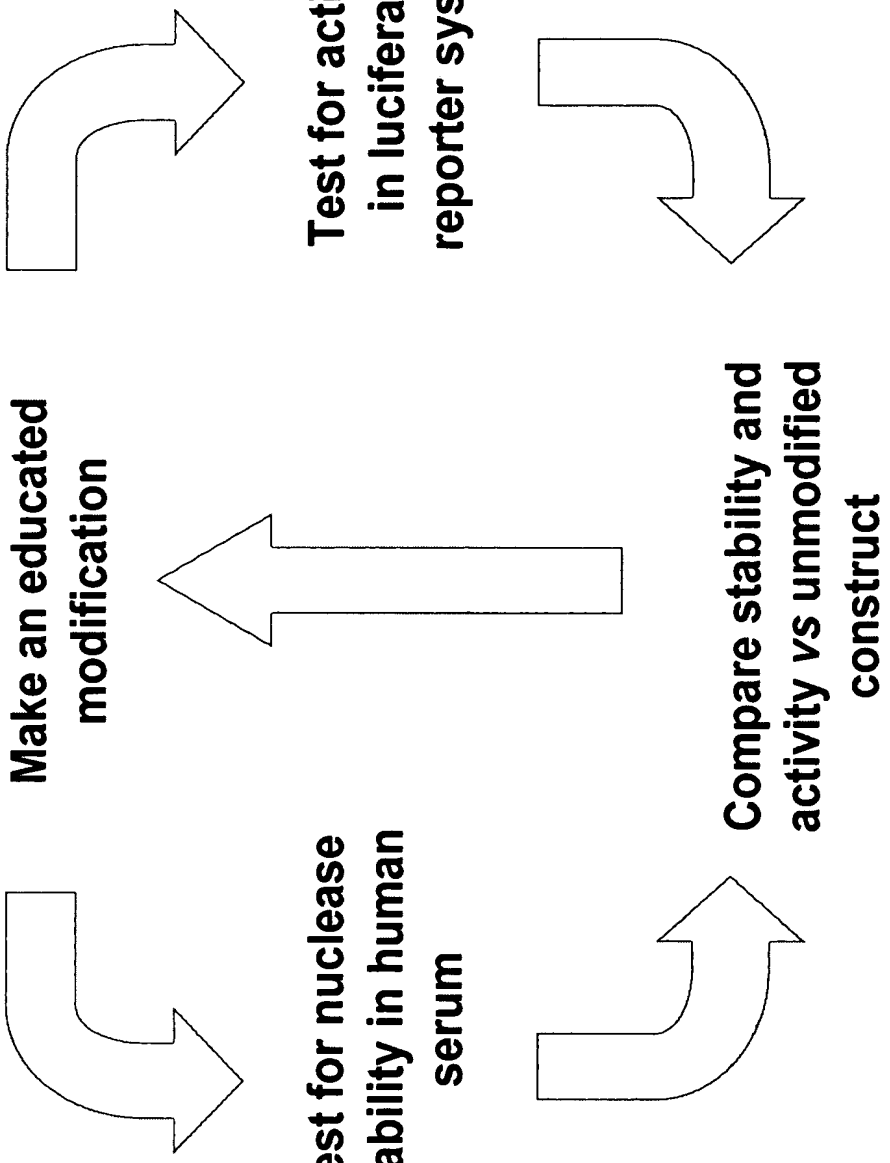
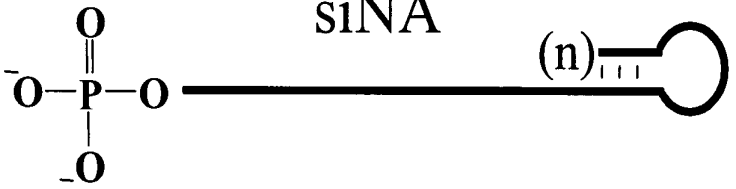
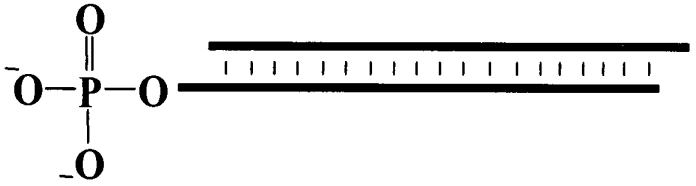


Figure 12: Phosphorylated siNA constructs

Phosphates can be modified as described herein

Asymmetric hairpin siNA





Asymmetric duplex siNA

(n) = number of base pairs (e.g. 3-18 bp)

Figure 13: 5'-phosphate modifications

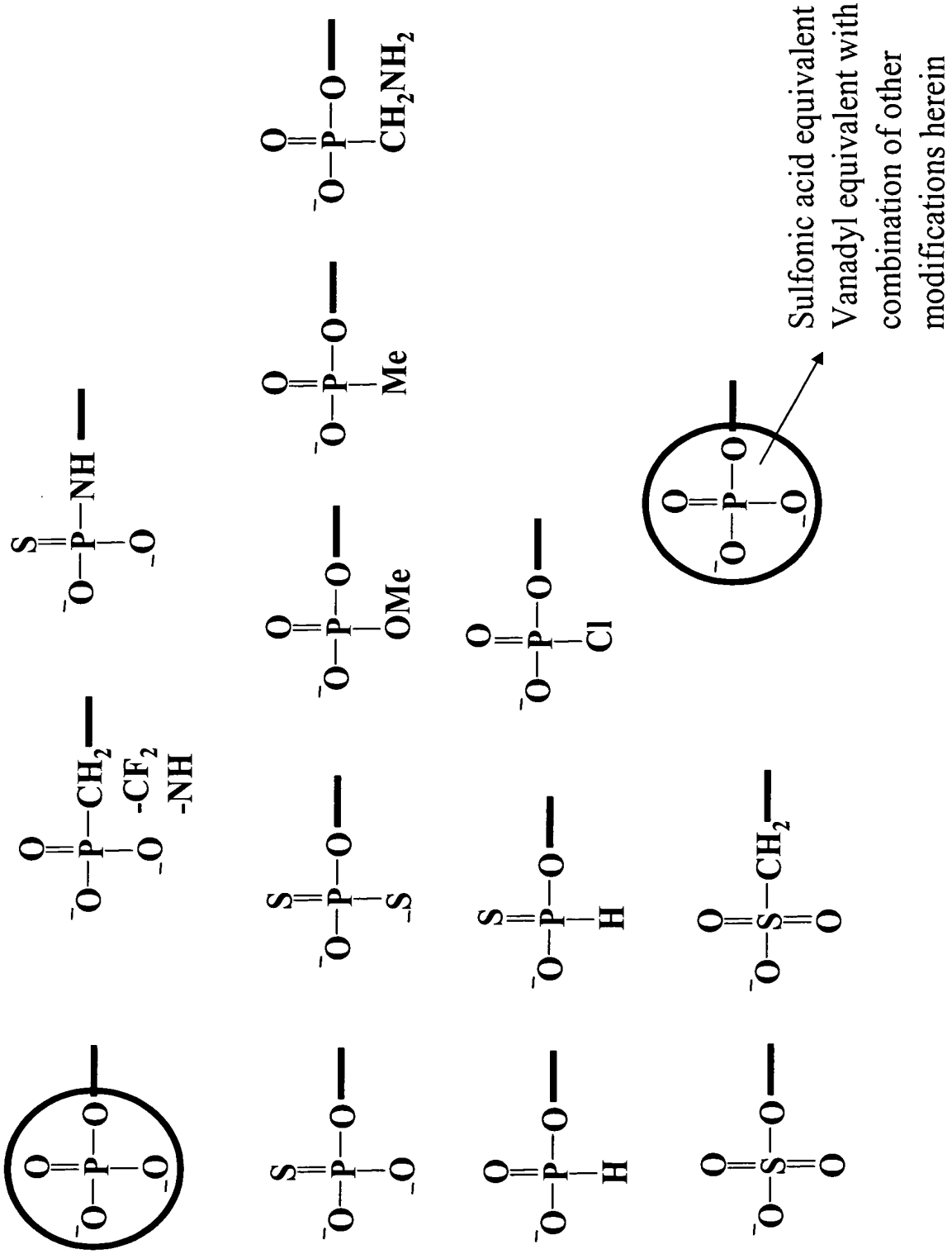


Figure 14A: Duplex forming oligonucleotide constructs that utilize palindrome or repeat sequences

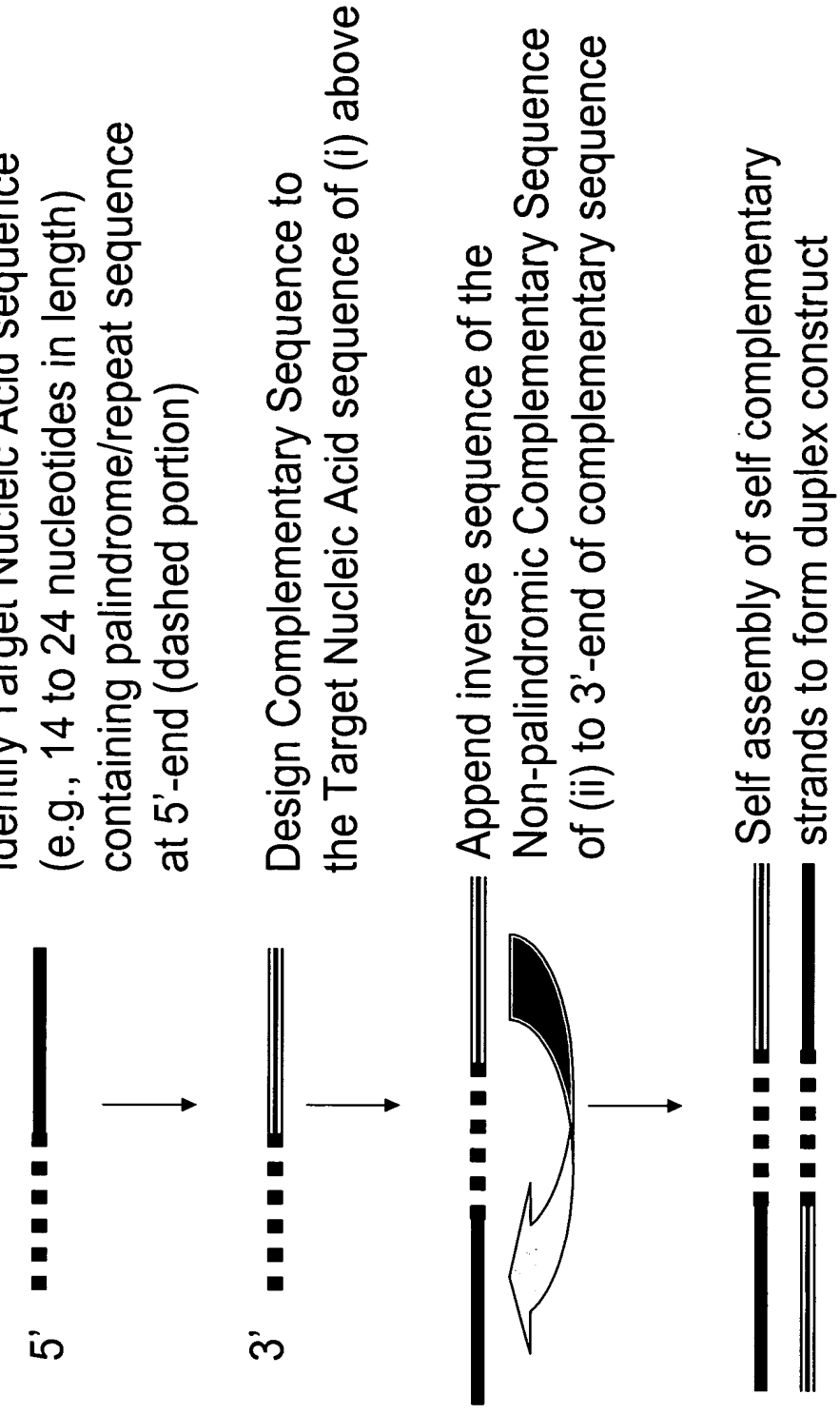
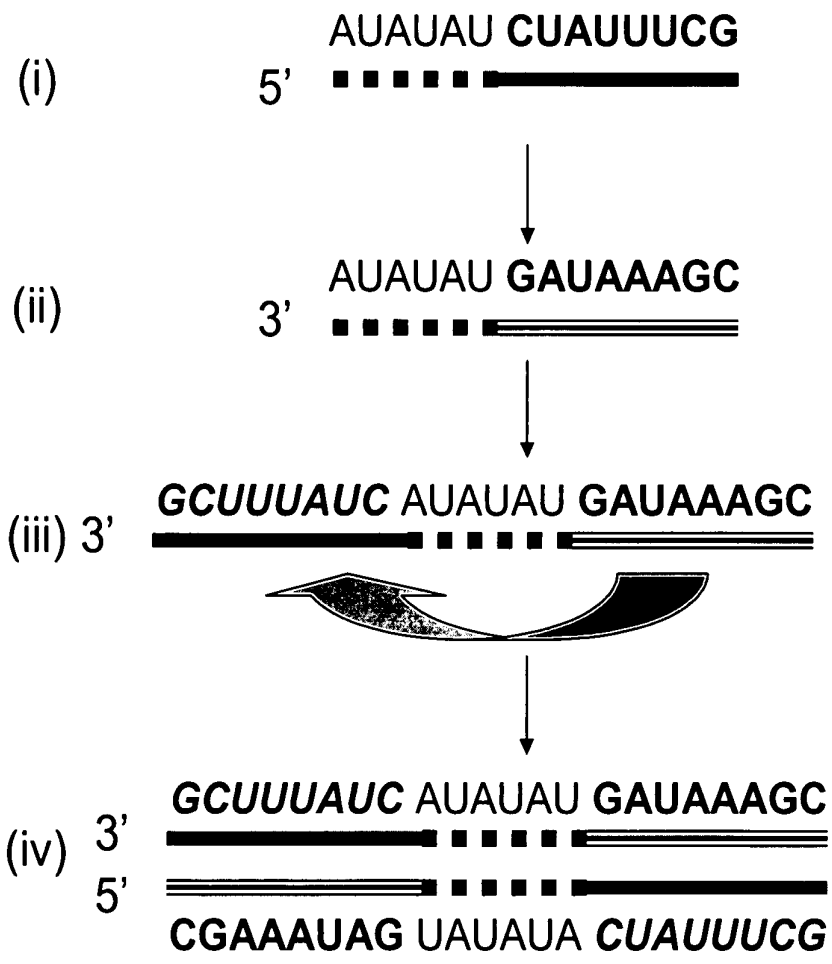


Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

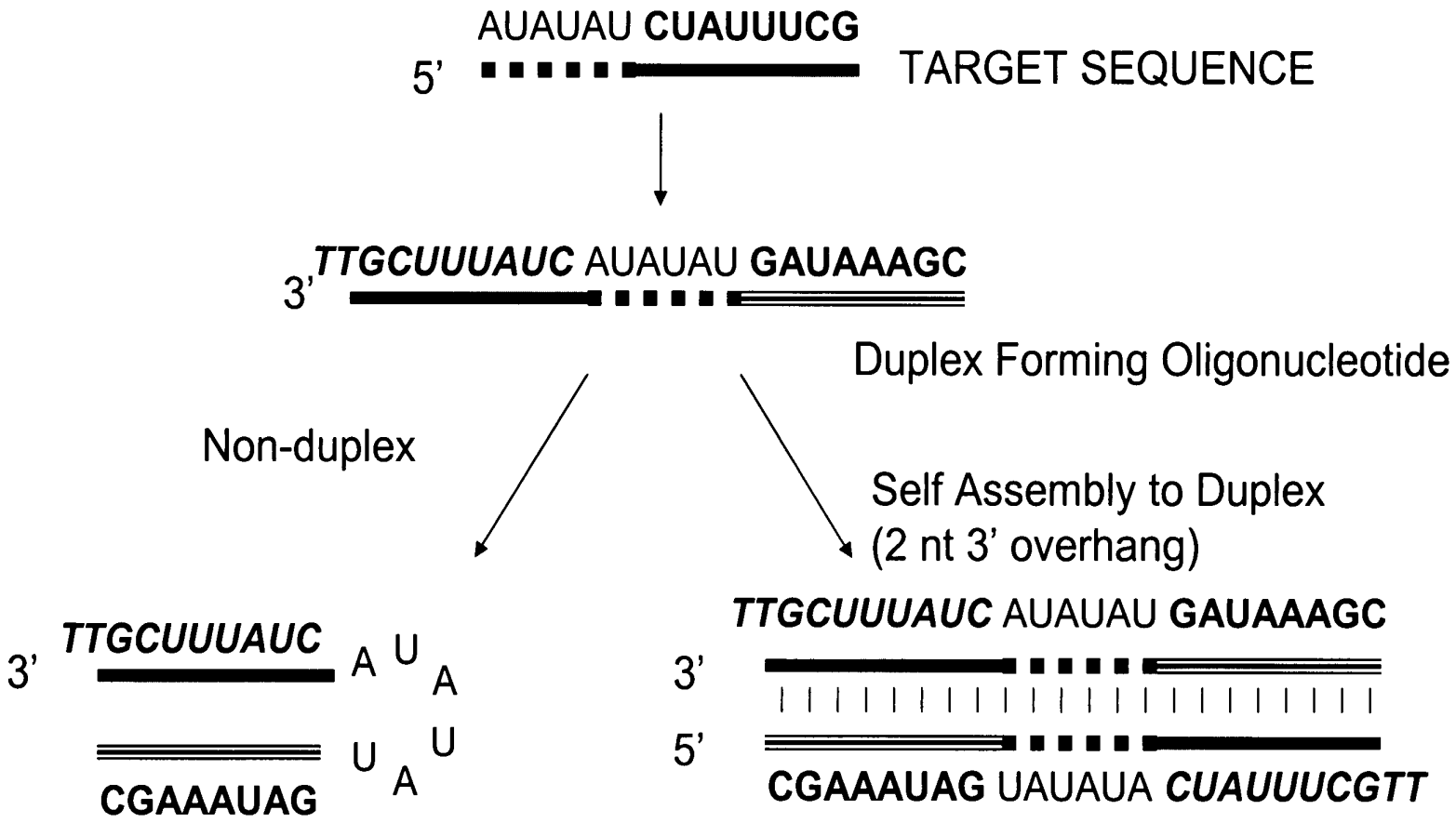


Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

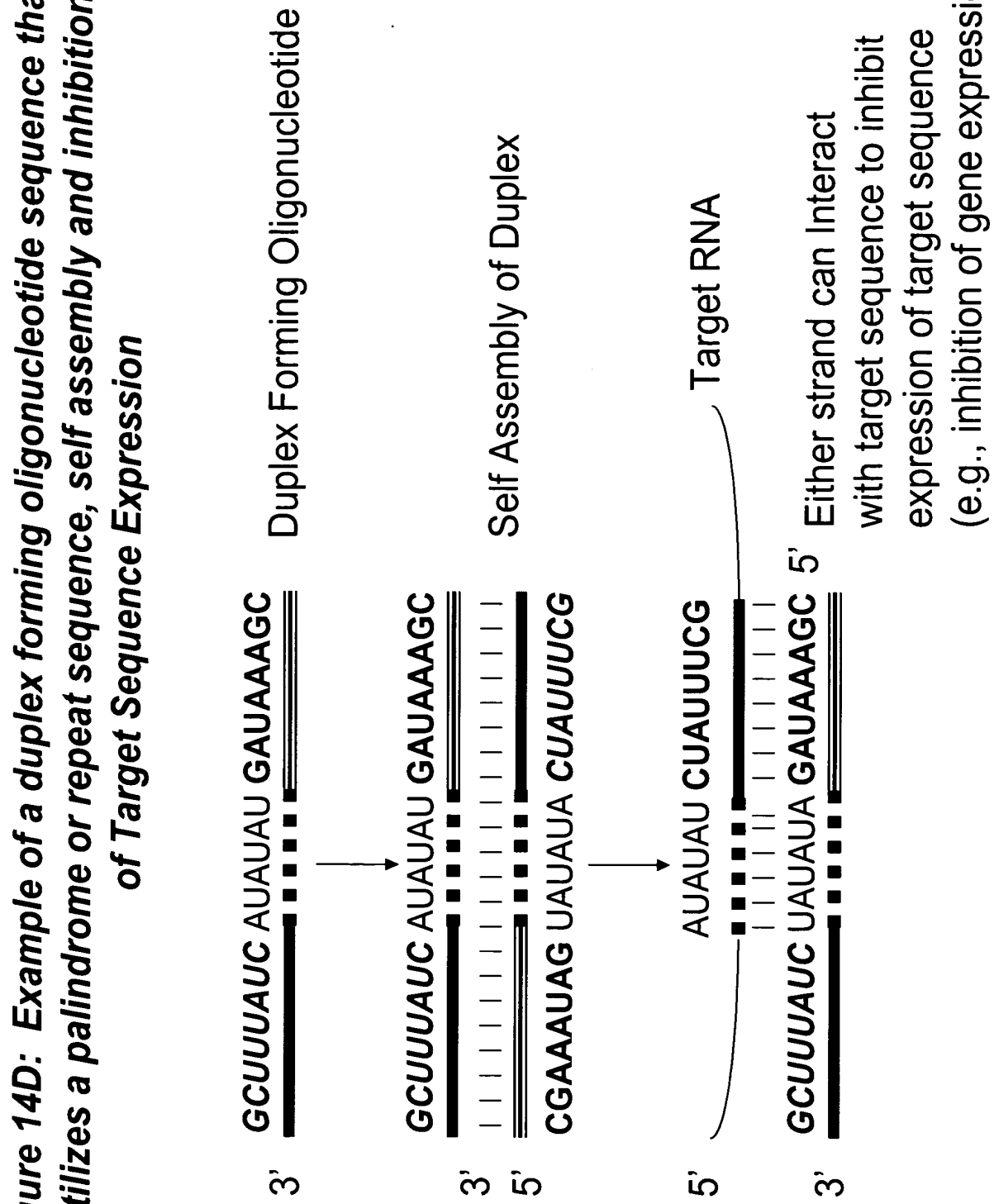


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences

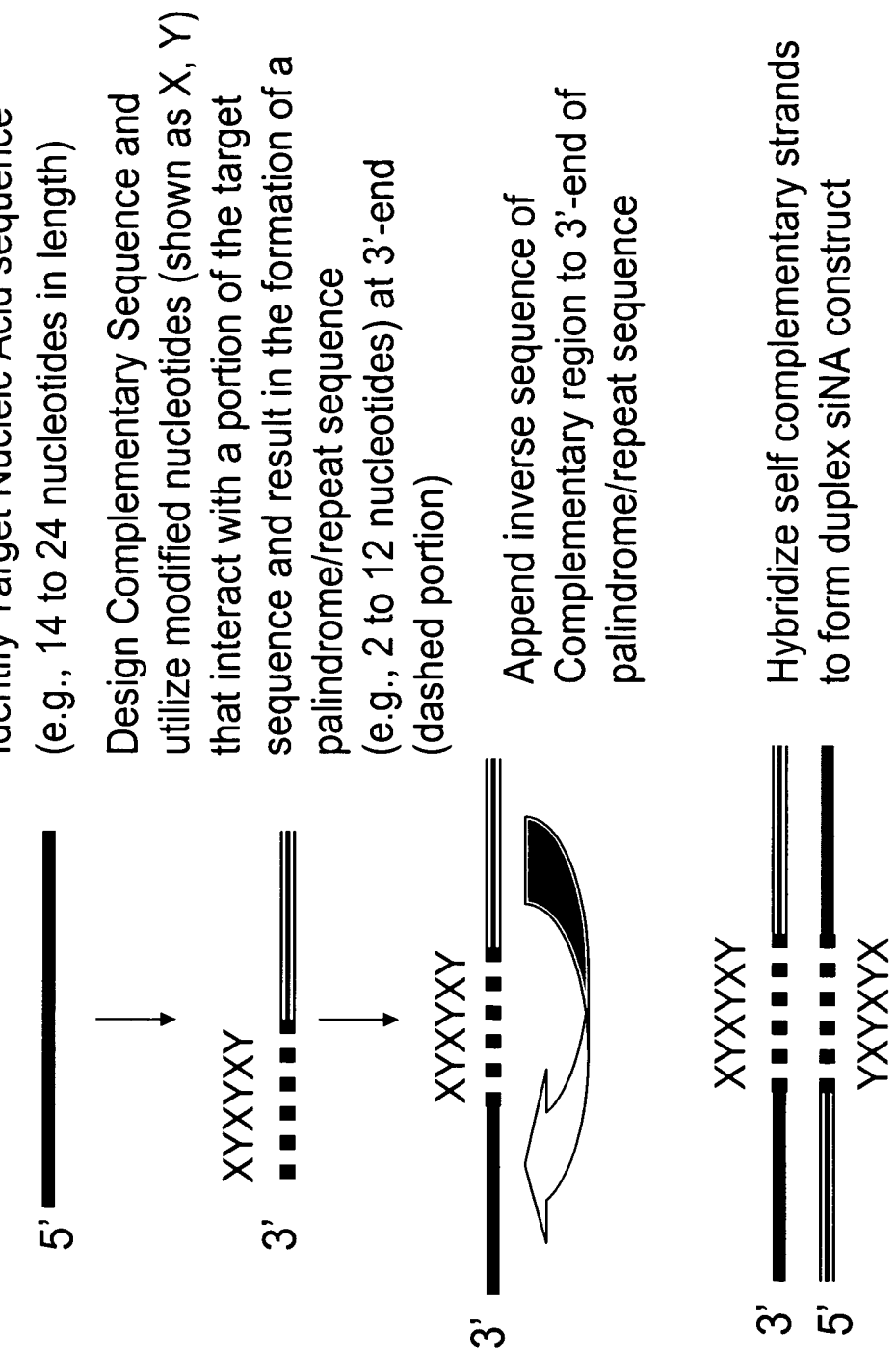


Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

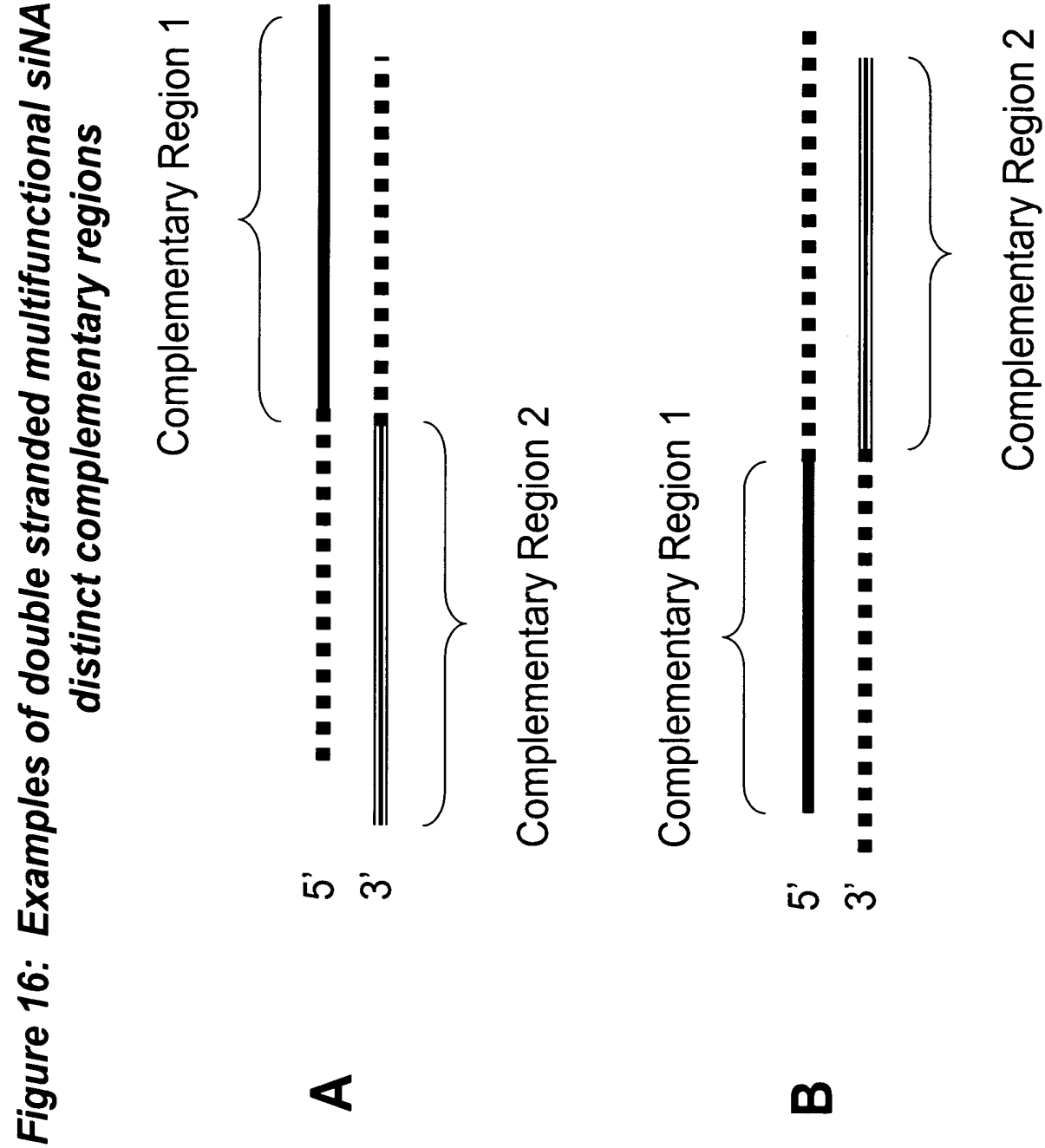


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

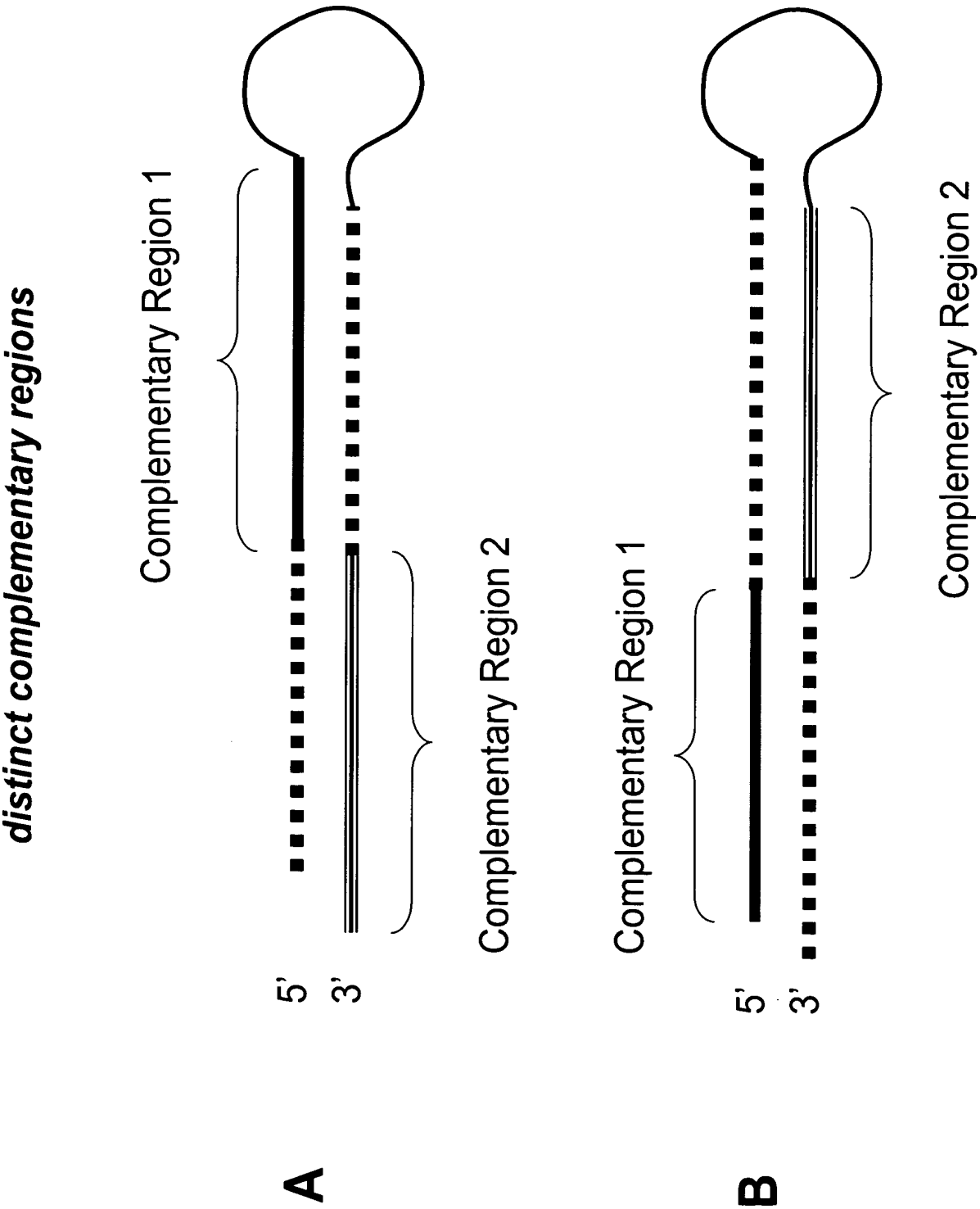


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

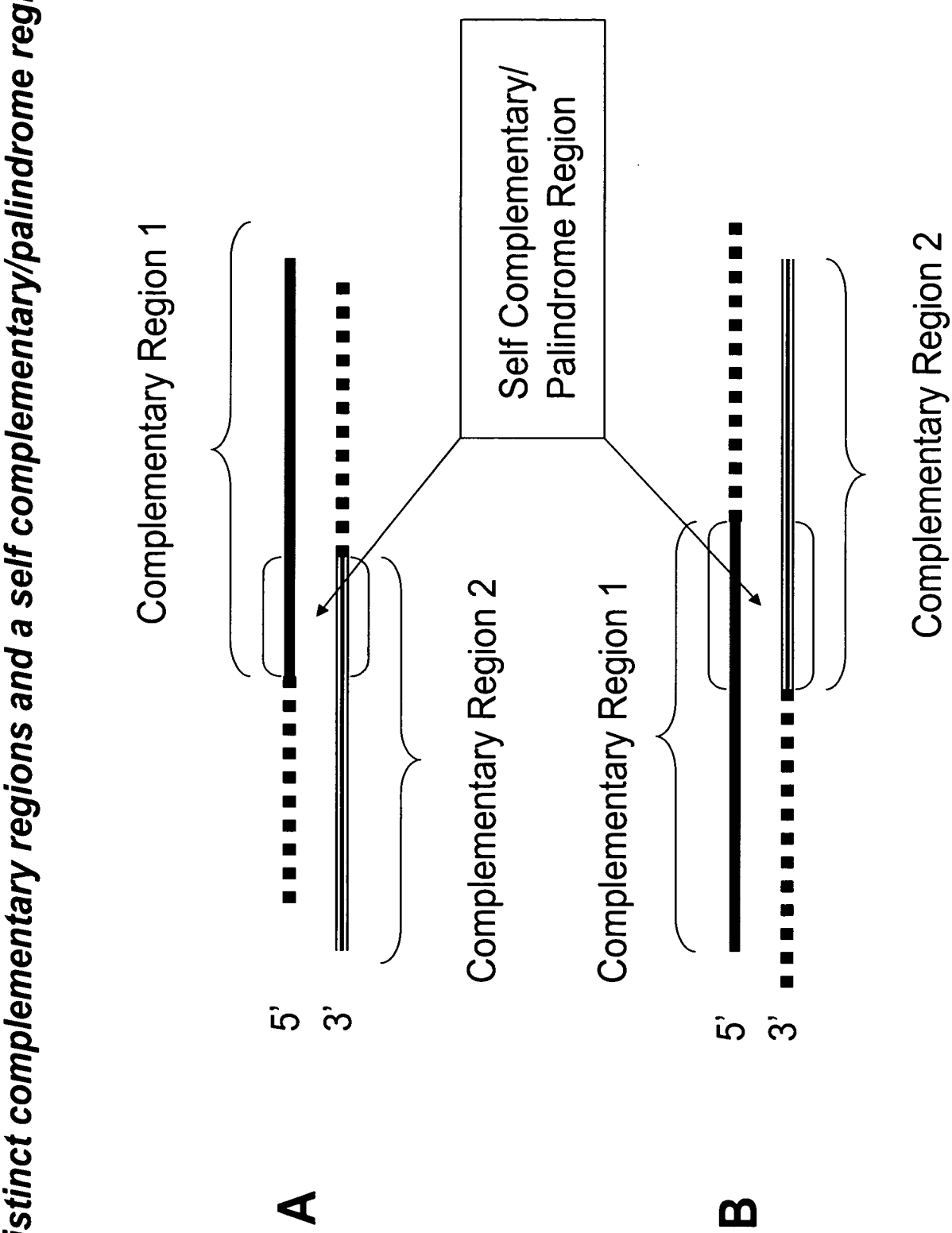


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

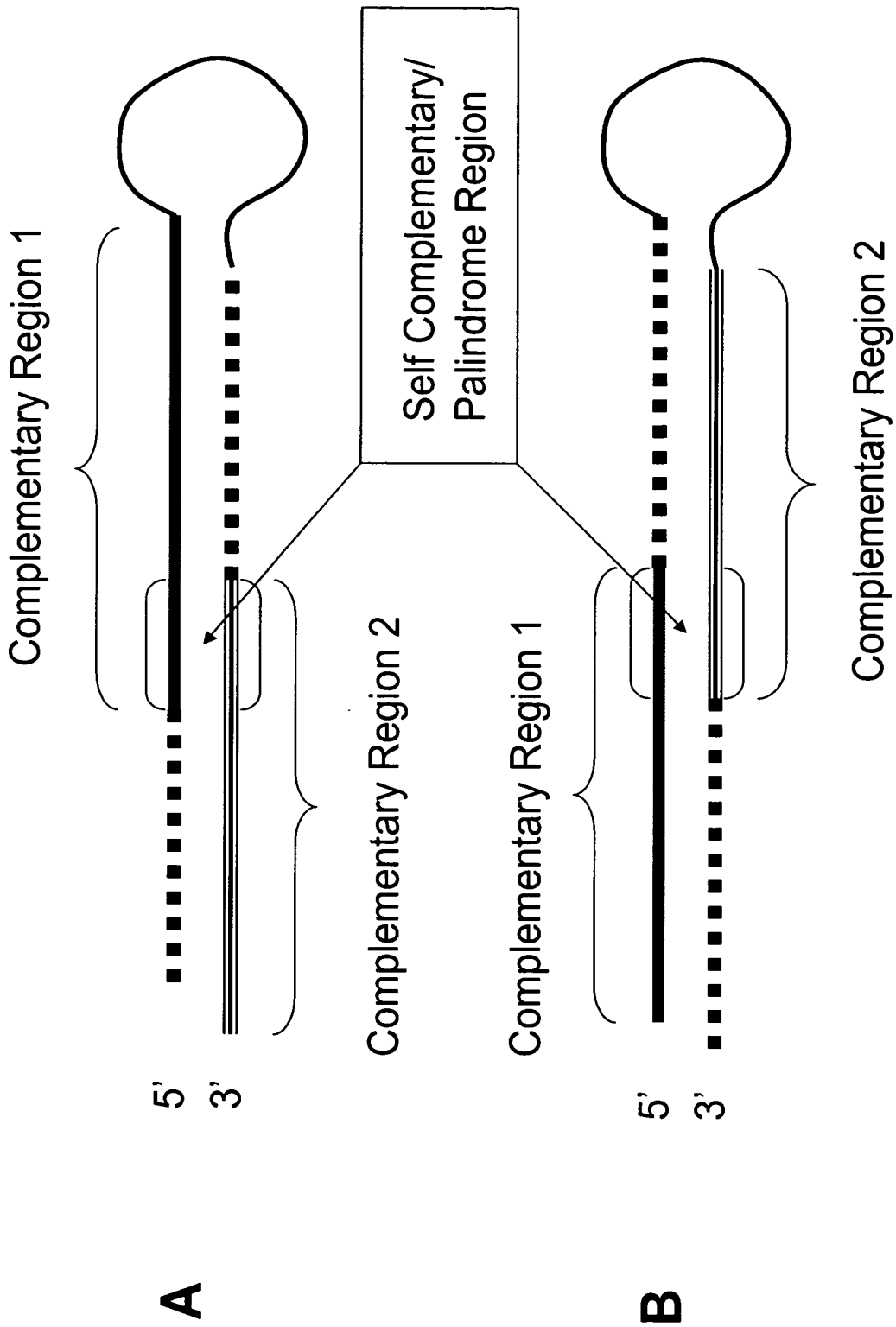


Figure 20: Example of multifunctional siNA targeting two separate Target nucleic acid sequences

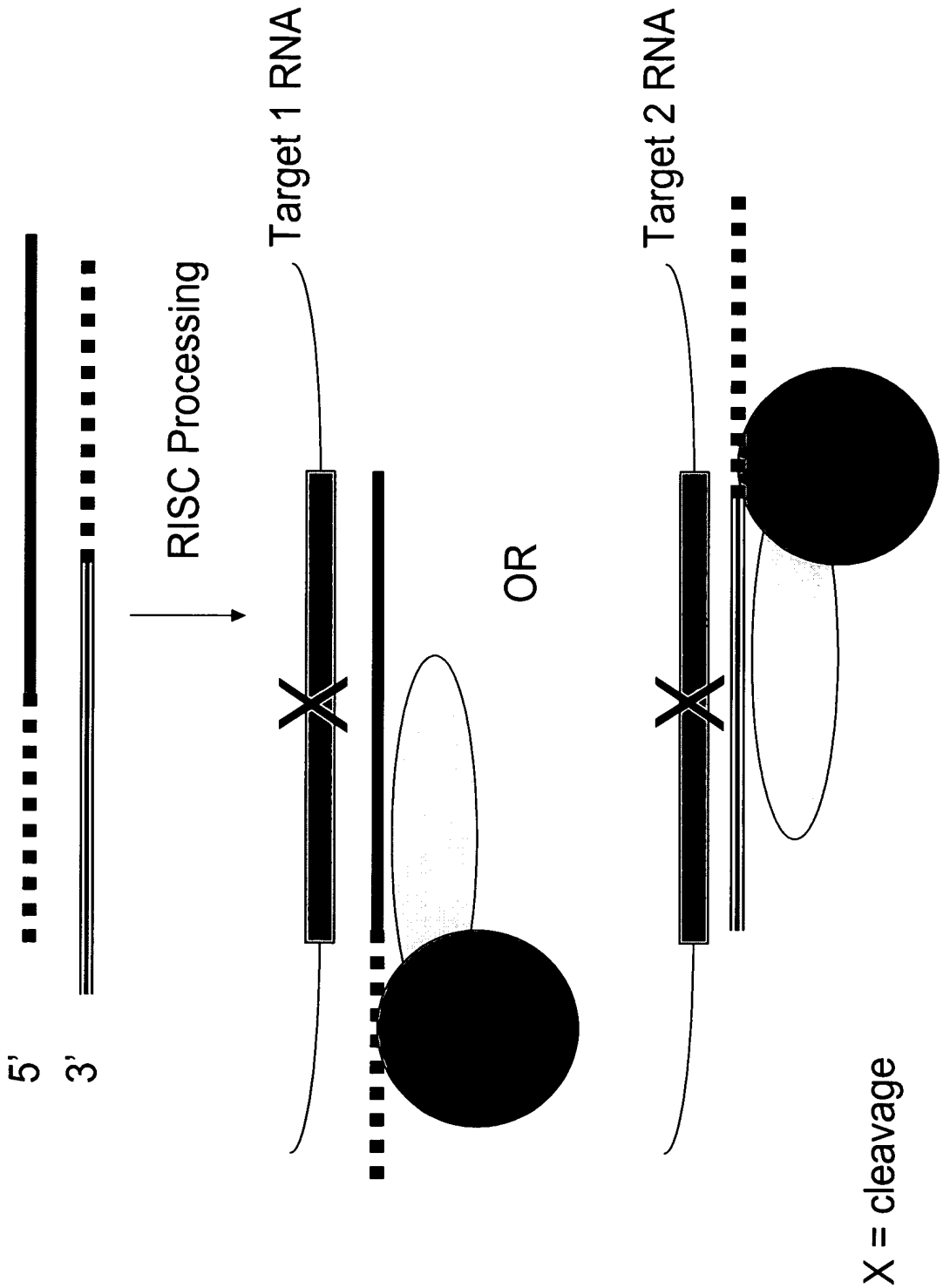


Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence